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THE COUNCIL FOR TOBACCO RESEARCH—U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JAN 29 1975

Application for Research Grant

Date: 1-24-75

(Use extra pages as needed).

1. Principal Investigator (give title and degrees):

John L. Egle, Jr., Ph.D.
Associate Professor of Pharmacology

2. Institution & address:

Medical College of Virginia
Department of Pharmacology
Box 726
Richmond, VA 23298

3. Department(s) where research will be done or collaboration provided:

Department of Pharmacology

4. Short title of study:

Respiratory retention and effects on pulmonary function of constituents of the vapor phase of cigarette smoke.

5. Proposed starting date: July 1, 1975

6. Estimated time to complete: Two years

7. Brief description of specific research aims:

1. Complete a study of the respiratory tract retention of constituents of the vapor phase of cigarette smoke.

   A. Study the effect of ventilatory rate, tidal volume and concentration inhaled on retention of these constituents.

   B. Study the retention of these compounds by the total respiratory tract and by the upper and lower portion.

2. Study the effects of all major vapor phase constituents on pulmonary function. Parameters to be measured will include:

   A. Ventilatory rate
   B. Tidal volume
   C. Pulmonary resistance
   D. Pulmonary compliance
   E. Transpulmonary pressure and intrapleural pressure as a means of determination of pulmonary resistance and pulmonary compliance.

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Compounds to be studied will include:

Part 1: Nitric oxide, hydrogen cyanide, methyl chloride, acetonitrile and furan.

Part 2: Acetaldehyde, formaldehyde, acrolein, isoprene, acetone, ammonia, methanol, benzene, toluene and the compounds indicated for study in Part 1.

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8. Brief statement of working hypothesis:

More than 50 compounds are known to occur in the vapor phase of cigarette smoke at a concentration of one microgram or more per 40 ml puff.[1] It is proposed that the respiratory tract retention be measured in experimental animals of compounds not previously studied in this manner and known to occur in relatively high concentrations or considered to be noxious substances. Examples of such substances include nitric oxide, hydrogen cyanide, methyl chloride, acetonitrile and compounds in the furan series. A second objective would be to systematically study the effects of these compounds, as well as those previously studied in terms of retention, on such parameters of respiratory function as rate, tidal volume, pulmonary resistance, pulmonary compliance, and intrapleural pressure. Such studies would permit comparison of the capacity of individual smoke constituents to alter respiratory function. Since the substances proposed for study are brought into contact with the respiratory tract during smoking, it would seem desirable to know as much as possible about their handling by the respiratory tract in terms of both total respiratory tract retention as well as fractional retention by subdivisions of the respiratory tract. It would also be useful to determine the concentrations of the substances actually reaching the lower tract when a given concentration is inhaled. We have obtained this type of information for several compounds.[2-7] More recently toluene and benzene have been studied.

~~9. Details of experimental design and procedures (append extra pages as necessary)~~

It would also appear very desirable to know more about the ability of these substances to alter pulmonary function. Aviado[8] and other investigators[9-10] have studied the effect of cigarette smoke on pulmonary resistance and compliance and have reported changes in these parameters. The experimental conditions and results of these studies have varied considerably. Considerable attention has been devoted to the effects of such major air pollutants as NO_2 and SO_2 on pulmonary function.[11-12] However, it appears that there has been no systematic effort to determine and compare the effects, if any, on pulmonary function of important individual constituents of cigarette smoke at concentrations which would be encountered during smoking. This type of study would also permit identification of the components responsible for any changes of this type attributed to whole cigarette smoke. The proposed study is designed to answer these questions.

9. Details of experimental design and procedures:

1. Respiratory retention of vapor phase constituents

Vapors of each pure substance dispersed in air will be studied individually. Concentrations approximating those reported for each compound in the gas phase of cigarette smoke will be employed. Appropriate amounts of each substance to give the desired concentration when diluted will be introduced into a 13.5 liter recording respirometer. The respirometer will contain mineral oil as an insulating substance and a fan will be inserted in the system for circulating the vapor. The compounds studied thus far have been quite stable in such a system, therefore it seems reasonable to assume it would be satisfactory for the other vapors. The animal will be anesthetized and connected to the system by means of a mask or endotracheal tube and permitted to inhale vapor from the respirometer. The direction of vapor flow will be controlled by two-way valves. The animal will inhale vapor from the respirometer and exhale into a collecting bag. Such a system keeps the concentration inhaled constant. By taking samples of the vapor in the respirometer and collecting bag to determine the concentration of each and by recording the volumes inhaled and exhaled, it is possible to calculate the amount retained and the percentage of that inhaled which is retained.

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Using this system it is possible to measure retention of the vapors being studied under varying conditions of ventilatory rate, tidal volume, and concentration inhaled. Efforts will constantly be made to ensure that there is no loss of vapor from the system which might erroneously be interpreted as respiratory tract uptake. This would include conducting sham experiments and making any necessary corrections in results obtained in inhalation experiments. This would include taking into account all dead space in the system.

In addition to retention of each vapor by the entire respiratory tract, another major aim will be to determine the amount taken up by different segments of the respiratory tract. This can be accomplished by exposing isolated portions of the respiratory tract. Upper tract retention will be measured by severing the trachea at its lower end and passing vapor through the nose using a face mask and collecting it from the trachea. Upper tract experiments will be conducted on a "one-way" or "two-way" basis. In the one-way experiments the vapor will be passed into a collecting bag at the lower end of the trachea. A two-liter syringe will be attached to the end of the trachea for the two-way experiments, with the vapor being drawn into the syringe, then returned upward and expelled through the nose, finally being collected in a bag after passing through a two-way valve.

The two-way upper tract experiments will be carried out to determine the amount of compound under investigation taken up by this region during a complete ventilatory cycle. The one-way upper-tract experiments do not simulate any type of normal ventilation. The purpose of these experiments will be to measure the reduction in concentration of the inhaled vapor between the nose and bronchioles. Information of this type would be of value in the design and interpretation of experiments involving nasal inhalation and also in studies dealing with effects on lower tract tissue using isolated organ or in vitro systems. The one-way experiments will indicate the amounts of any substance removed by the upper tract and also provide a determination of the concentration actually reaching the lower tract. The two-way experiments would permit measurement of the amount taken up by this portion of the respiratory tract during a complete ventilatory cycle.

Lower tract retention will be measured by inserting an endotracheal tube thereby restricting the area of exposure to the lower portion of the respiratory tract. Otherwise, the techniques are similar to the total tract experiments.

All of these experiments will be of short duration, 1-3 minutes, and will involve the inhalation of a total of 1-3 liters of the vapor being studied.

2. Effects of vapor phase constituents on pulmonary function

Procedures for measurement of pulmonary function

Ventilatory rate, tidal volume, and minute volume are simple but important aspects of pulmonary function that can be easily measured with a respirometer.

To measure pulmonary resistance and pulmonary compliance, we will utilize a modified exposure chamber to simulate a plethysmograph. A schematic diagram of the apparatus is shown in figure 1. The dogs will be anesthetized with

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30 mg/kg pentobarbital sodium. An endotracheal tube will be installed to limit airway exposure to the mouth opening only. Placed onto the endotracheal tube will be an adaptor fixed by a connector. The distal side of the adaptor will be attached to a pneumotachograph connected to a differential transducer (1) to measure air flow ($\dot{v}$)(pressure drop across the pneumotachograph). Into the right interpleural space a rubber catheter will be permanently secured. The opening of the catheter will be connected to a Statham differential transducer (2). This transducer will also be connected to a lead from the adapter. The catheter will be used to measure the intrapleural pressure, the adapter used to measure the open or mouth airway pressure. These two pressure readings will be, with the use of the transducer, represented as one reading known as the transpulmonary pressure. A third transducer (3) will be used to measure the pressure differential between the chamber and the ambient air. This measurement is a component of pulmonary resistance. The signals from the three transducers will be fed into a Tektronix S103N oscilloscope. For our purposes, we will have three channels in operation, two horizontal and one vertical. A picture of each set of experiments will be taken and developed. The pictures will be used to plot the tangent of the graphs. From the loops on the oscilloscope the pulmonary resistance and compliance can be calculated using the tangent of the loop over its linear range by the method described by Mead and Wittenburg.[13] Measurement of end tidal CO_2 levels will also be made during these experiments.

Acute inhalation

The animals will be prepared for measurement of pulmonary function as described. Vapors of each compound to be studied will be generated by passing air through the substance. This concentration vapor will be transferred to a recording respirometer. The respirometer will serve as a reservoir for the vapor and also permit monitoring of the respiratory pattern during the exposure period. The animals will inhale the vapor for a varying period ranging from one inhalation to at least three minutes. (Aviado[8] has reported that one puff of cigarette smoke is sufficient to produce an immediate increase in pulmonary resistance.) Depending upon the initial experience (response or lack of response) with each compound, the exposure period will be adjusted to obtain a clear picture of the relationship between exposure time and alteration of pulmonary function for each substance.

Chronic inhalation

For these experiments, dogs will be placed in large (approximately 300 liter) chambers and exposed to each of the vapors being investigated. Exposure will be based on the assumption that a heavy smoker would be exposed to approximately 400 inhalations of smoke (and therefore the chemicals being studied) each day. Thus, the dogs will be allowed to inhale the vapor about 400 times or for a period of 20 to 30 minutes (depending on the ventilatory rate). Dogs will be exposed 5 days a week. Shorter or longer periods of exposure may also be selected depending upon the results with each compound. A dynamic system will be used for exposure. A vapor of the compound to be studied will be generated by continuous air flow through the substance. This vapor will then be mixed with air to achieve the proper concentration as it flows into a reservoir. The vapor will be drawn from the reservoir through the exposure chamber at a constant flow

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rate. A negative pressure system will be employed with a vacuum pump pulling the vapor into the chambers with flow controlled by a critical orifice. The concentration of the compound being studied in the chamber will be monitored by periodic sampling and analysis by gas chromatography.

Periodically, each animal will be placed in the plethysmograph and measurements of pulmonary function performed. Such tests will be carried out on each animal at 7 day intervals during the exposure period. Animals will continue to be tested after the period of exposure, particularly if alterations in pulmonary function are observed and if they persist beyond the period of exposure. Thus, the duration of any changes produced under these conditions will be determined or it will be established that they persist until a certain point after which measurements are no longer made (e.g., six months). It would appear that the exact design of these chronic experiments, particularly in regard to duration, would depend upon the magnitude and duration of effects observed with each compound.

Animals

It is anticipated that all experiments will be performed with mongrel dogs. Such animals have been used in the studies carried out involving respiratory retention of vapor phase constituents. Little variability has been observed between dogs for a given set of experimental conditions and it has been possible to pool the data for all animals within a particular experimental series. It is conceded that there would be some advantages in using purebred beagles, but these animals would be approximately ten times more expensive and it is doubted that the benefits of using such animals outweigh the considerable cost involved.

Chemical Analysis

The compounds studied thus far have been measured by either spectrophotometry (all aldehydes, acetone and ammonia) or gas chromatography (isoprene, methanol, benzene, toluene and furan). The fact that we have experience with and established methodology for measuring very low concentrations of these compounds would facilitate the initiation of experiments involving tests of pulmonary function. Possibly all of these compounds would be analyzed by gas chromatography in the future. The compounds not previously studied would also be measured by gas chromatography although techniques (selection of column, etc.) would have to be devised for them.

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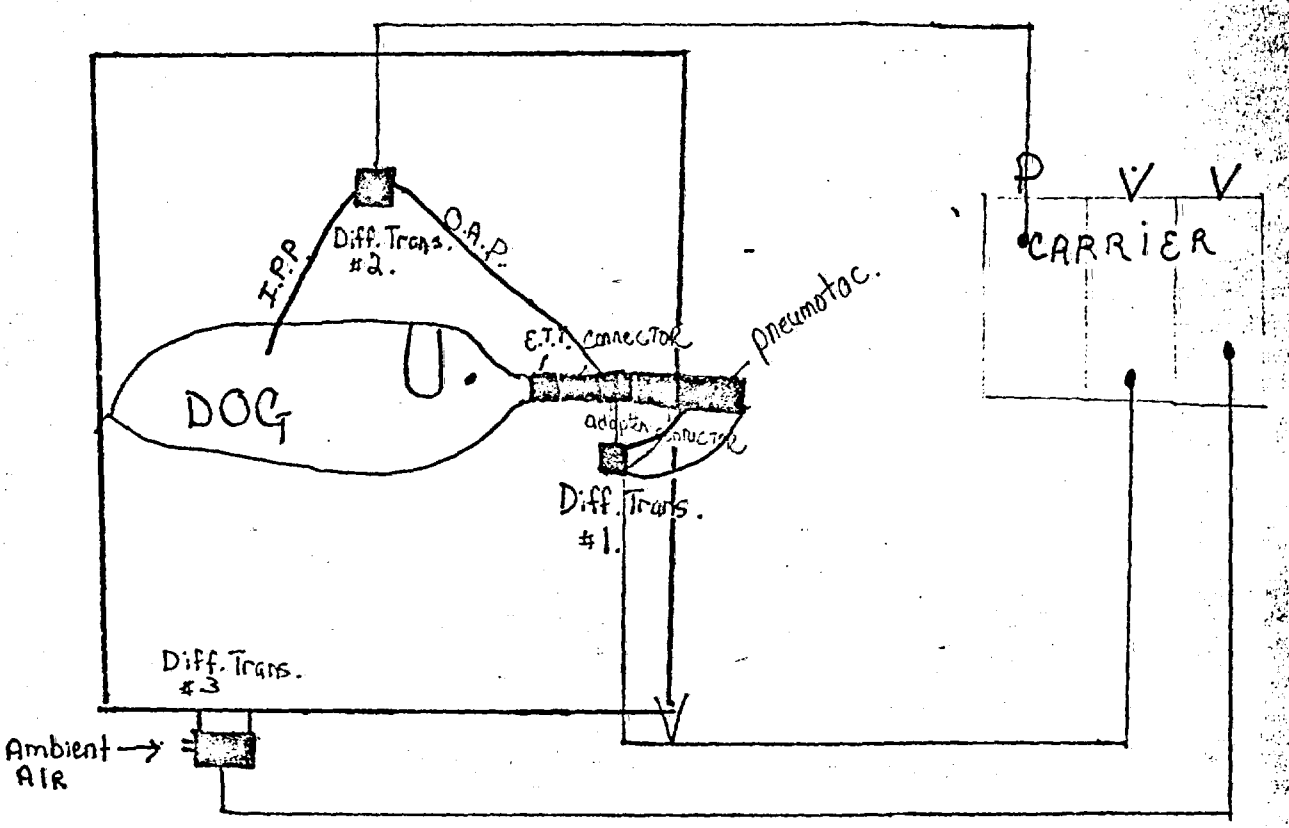
Fig 1



I.P.P. - Intra pleural pressure
E.T.T. - Endotracheal tube
O.A.P. - Open Airway Pressure
pneumotac - Pneumotachograph
Diff. Trans. - Differential transducer

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References

1. Newsome, J. R., Norman, V. and Keith, C. H.: Vapor phase analysis of tobacco smoke. Tobacco Sci. 9:102-110, 1965.

2. Egle, J. L., Jr.: Retention of inhaled acetaldehyde in man. J. Pharmacol. Exp. Ther. 174:14-19, 1970.

3. Egle, J. L., Jr.: Retention of inhaled acetaldehyde in the dog. Arch. Environ. Health 24:354-57, 1972.

4. Egle, J. L., Jr.: Retention of inhaled formaldehyde, propionaldehyde and acrolein in the dog. Arch. Environ. Health 25:119-124, 1972.

5. Egle, J. L., Jr.: Retention of inhaled acetone and ammonia in the dog. Amer. Ind. Hyg. Asso. J. 34:533-39, 1973.

6. Egle, J. L., Jr.: Respiratory retention of isoprene and methanol in the dog. Toxicol. Appl. Pharmacol. 25:470-471, 1973.

7. Egle, J. L., Jr. and Gochberg, B.: Respiratory retention of toluene and benzene in the dog. The Pharmacologist 16:299, 1974.

8. Aviado, D. M. and Palecek: Pulmonary effects of tobacco and related substances I. Pulmonary compliance and resistance in the anesthetized dog. Arch. Environ. Health 15:187-193, 1967.

9. Eich, R. H., Gilbert, R. and Auchincloss, J. H., Jr.: The acute effect of smoking on the mechanics of respiration in chronic obstructive pulmonary emphysema. Amer. Rev. Tuberc. 76:22-23, 1957.

10. Atlinger, E. O., Goldstein, M. M. and Segul, M. S.: Effects of smoking upon the mechanics of breathing. Amer. Rev. Tuberc. 77:10-16

11. Lewis, T. R., Campbell, K. I. and Vaughan, T. R., Jr.: Effects on canine pulmonary function. Arch. Environ. Health 18:596, 1969.

12. Vaughan, T. R., Jr., Jennelle, L. F. and Lewis, T. R.: Long-term exposure to low levels of air pollutants: Effects on pulmonary function in the beagle. Arch. Environ. Health 19:45-50, 1969.

13. Mead, J. and Whittenberger, J. L.: Physical properties on human lung measured during spontaneous respiration. J. Appl. Physiol. 5: 779-796, 1953.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Facilities for conducting this study are available in the Department of Pharmacology. This includes laboratories for performing the experiments and animal quarters. Available equipment includes a Beckman GC 72-5 gas chromatograph, a Beckman DU-2 spectrophotometer, two Collins 13.5 liter respirometers, a Harvard respirator, two exposure chambers, a Tektronix S103N oscilloscope, an Instrumentation Association Capnograph, and a six channel Grass Model 7 polygraph.

11. Additional facilities required:

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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12. Biographical sketch of investigator(s) and other professional personnel:

NAME: John Lee Egle, Jr.

BIRTH DATE: July 13, 1939

BIRTH PLACE: Martinsburg, West Virginia

CITIZENSHIP: United States

SOCIAL SECURITY NUMBER: [redacted]

MARITAL STATUS: Married; Two Children

HOME ADDRESS: 8114 Duncaster Road, Richmond, Virginia 23235

OFFICE ADDRESS: Department of Pharmacology, Medical College of Virginia
Richmond, Virginia 23298

EDUCATION:

B.S.	Shepherd College	Shepherdstown, West Virginia	1961
M.S.	West Virginia University	Morgantown, West Virginia	1963
Ph.D.	West Virginia University	Morgantown, West Virginia	1964

POSTDOCTORAL TRAINING:

Cardiovascular Training Program - Bowman Gray School of Medicine
Winston-Salem, North Carolina (1964-1966)

ACADEMIC APPOINTMENTS:

Department of Pharmacology Medical College of Virginia Richmond, Virginia 23298	Assistant Professor	1966-1974
Department of Pharmacology Medical College of Virginia Richmond, Virginia 23298	Associate Professor	1974-

MEMBERSHIP-SCIENTIFIC SOCIETIES:

ASPET

Society of Toxicology

Sigma Xi

AAAS

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PUBLICATIONS

Egle, John L., Jr., Meredith, Jessee H. and Little, J. Maxwell:

The effect of dichloroisoproterenol, pronethalol and propranolol on oxygen consumption in the rat.
J. Pharmacol. Exp. Ther. 158: 399-404, 1967.

Meredith, Jessee H., Little, J. Maxwell and Egle, John L., Jr.:

A comparison of simultaneous cardiac output measurements by two methods: the Oxygen-Fick principle and the electromagnetic flow-meter.
Amer. Surgeon 33: 949-52, 1967.

Egle, John L., Jr.:

Retention of inhaled acetyladehyde in man.
J. Pharmacol. Exp. Ther. 174: 14-19, 1970.

Egle, John L., Jr.:

Single breath retention of acetaldehyde in man.
Arch. Environ. Health 23: 427-433, 1971.

Egle, John L., Jr.:

Retention of inhaled acetaldehyde in the dog.
Arch. Environ. Health 24: 354-357, 1972.

Egle, John L., Jr.:

Retention of inhaled formaldehyde, propionaldehyde and acrolein in the dog.
Arch. Environ. Health 25: 119-124, 1972.

Egle, John L., Jr.:

Effects of inhaled acetaldehyde and propionaldehyde on blood pressure and heart rate.
Toxicol. Appl. Pharmacol. 23: 131-135, 1972.

Egle, John L., Jr., Putney, James W., Jr. and Borzelleca, Joseph F.:

Effects of haloalkane propellants on cardiac rate and rhythm in mice.
JAMA 222: 786-789, 1972.

Egle, John L., Jr., Hudgins, Patricia M., and Lai, Fong M.:

Cardiovascular effects of intravenous acetaldehyde and propionaldehyde in the anesthetized rat.
Toxicol. Appl. Pharmacol. 24: 636-643, 1973.

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Source: https://www.industrydocuments.ucsf.edu/docs/zqdm0000

PUBLICATIONS

Egle, John L.,Jr.:

Retention of inhaled acetone and ammonia in the dog.
Amer. Ind. Hyg. Ass. J. 34:533-39, 1973.

Egle, John L.,Jr. and Hudgins, Patricia M.:

Dose dependent sympathomimetic and cardioinhibitory effects of acrolein and formaldehyde in the anesthetized rat.
Toxicol. Appl. Pharmacol. 28:358-66, 1974.

Beckner, Jacqueline S., Hudgins, Patricia M. and Egle, John L.,Jr.:

Effects of acetaldehyde, propionaldehyde, formaldehyde and acrolein on contractility, ^{14}C-norepinephrine and ^{45}Calcium binding in isolated smooth muscle.
Res. Commun. Chem. Pathol. Pharm. 9:471-88, 1974.

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Source: https://www.industrydocuments.ucsf.edu/docs/zqdm0000

ABSTRACTS

Egle, John L., Jr.:

Egle, J. L., Jr. and Saxe, L. H.:

Quantitative changes in the cecal flora and fauna of rats treated with antibiotics.
J. Parisitol. 49: 16, 1963.

Egle, J. L., Jr.:

Quantitative studies of antibiotic action upon the cecal flora and fauna of rats.
Fed. Proc. 23: 512, 1964.

Egle, J. L., Jr.:

Retention of inhaled acetaldehyde in man.
The Pharmacologist 10: 174, 1968.

Egle, J. L., Jr.:

Single breath human retention of inhaled acetaldehyde.
The Pharmacologist 12: 265, 1970.

Egle, J. L., Jr.:

Respiratory uptake of acetaldehyde and propionaldehyde in the dog.
The Pharmacologist 13: 273, 1971.

Hudgins, P. M., Egle, J. L., Jr. and Lai, F. M.:

Cardiovascular effects of acetaldehyde.
The Pharmacologist 13: 298, 1971.

Egle, J. L., Jr., Putney, J. W., Jr. and Borzelleca, J. F.:

Cardiac function in mice following exposure to haloalkane propellants alone and in combination with bronchodilators.
Toxicol. Appl. Pharmacol. 22: 306, 1972.

Egle, J. L., Jr.:

Respiratory uptake of formaldehyde, propionaldehyde and acrolein in the dog.
Fed. Proc. 31: 518, 1972.

Egle, J. L., Jr.:

Pressor effects of four aliphatic aldehydes and their interactions with ^{14}C-norepinephrine in an isolated smooth muscle preparation.
Fed. Proc. 32: 795, 1973.

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Source: https://www.industrydocuments.ucsf.edu/docs/zqdm0000

ABSTRACTS

Egle, J. L., Jr.:

Respiratory retention of acetone and ammonia in the dog.
Toxicol. Appl. Pharmacol. 25: 470-471, 1973.

Egle, J. L., Jr.:

Respiratory retention of isoprene and methanol in the dog.
Fed. Proc. 33: 569, 1974.

Egle, J. L., Jr. and Gochberg, B.:

Respiratory retention of toluene and benzene in the dog.
The Pharmacologist 16: 299, 1974.

Green, M. A. and Egle, J. L., Jr.:

LD_{50} determination and hypotensive effect of 3-methyl-2-benzothiazolone hydrazone.
Toxicol. Appl. Pharmacol. (in press)

Egle, J. L., Jr., Borzelleca, J. F. and Long, J.E.:

An evaluation of the cardiac sensitizing potential of Scotchgard.
Toxicol. Appl. Pharmacol. (in press)

Green, M. A. and Egle, J. L., Jr.:

Effects of intravenous acetaldehyde, acrolein, formaldehyde and propionaldehyde on arterial blood pressure following acute guanethidine treatment.
Fed. Proc. (in press)

Gochberg, B. J. and Egle, J. L., Jr.:

Retention of inhaled furan in the dog.
Fed. Proc. (in press)

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13. Publications: (five most recent and pertinent of investigator).

Egle, John L., Jr.:
Retention of inhaled acetyladehyde in man.
J. Pharmacol. Exp. Ther. 174: 14-19, 1970.

Egle, John L., Jr.:
Retention of inhaled acetaldehyde in the dog.
Arch. Environ. Health 24: 354-357, 1972.

Egle, John L., Jr.:
Retention of inhaled formaldehyde, propionaldehyde and acrolein in the dog.
Arch. Environ. Health 25: 119-124, 1972.

Egle, John L., Jr., Hudgins, Patricia M., and Lai, Fong M.:
Cardiovascular effects of intravenous acetaldehyde and propionaldehyde in the anesthetized rat.
Toxicol. Appl. Pharmacol. 24: 636-643, 1973.

Egle, John L., Jr.:
Retention of inhaled acetone and ammonia in the dog.
Amer. Ind. Hyg. Asso. J. 34: 533-539, 1973.

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14. First year budget:

A. Salaries (give names or state "to be recruited")	% time	Amount
Professional (give % time of investigator(s) even if no salary requested)		
John L. Egle, Jr., Ph.D. Associate Professor of Pharmacology	50	
Technical		
Bethe Gochberg Lab Specialist B	100	$10,512
Mark Green Lab Technician	50	4,000
Benefits		1,460
Sub-Total for A		$15,972

B. Consumable supplies (by major categories)	
Chemicals	500
Glassware	500
Animals	2,500
Animal Care	1,250
Sub-Total for B	4,750

C. Other expenses (itemize)			
Travel	750	Laundry	50
Slides & illustrations	200	Postage	50
Page charges and reprints	300		
Instrument repair & Maintenance	200		
Sub-Total for C			1,550
Running Total of A + B + C			$22,272

D. Permanent equipment (itemize)	
Exposure chambers	3,000
3 differential transducers	1,000
Pneumotachograph	200
Sub-Total for D	4,200
E	3,341
Total request	$29,813

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	$17,118	5,000	1,500		3,543	27,161
Year 3						

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Respiratory tract retention of vapor phase constituents of cagarette smoke	AMA-ERF	$67,068	November 1, 1972 October 31, 1975

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name John L. Egle, Jr.

Signature John L. Egle, Jr. Date 1-24-75

Telephone 804 770-4698
Area Code Number Extension

Checks payable to

Raymond T. Holmes, Jr.

Mailing address for checks

Mr. Raymond T. Holmes, Jr., Vice-Pres. Finance
1200 East Broad Street, Richmond, VA 23298

Responsible officer of institution

Typed Name Dr. John J. Salley

Title Asso. V-P for Research & Grad. Affairs

Signature John J. Salley Date 27 Jan 75

Telephone 804 770-5608
Area Code Number Extension

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